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ORIGINAL ARTICLE

Acetylcholine Release in Rapid Synapses

Two Fast Partners—Mediatophore and Vesicular Ca^{2+}/H^{+} Antiport

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Introduction

Rapid neurotransmission is like lightning: a spark of calcium in the nerve terminal, a spark of transmitter in the cleft, and the signal is over. But "time is gained at the expense of sensitivity" (Katz, 1988); transmission relies on low-affinity, high-speed reactions. These fast processes are modulated by regulating reactions that do not need to be so rapid.

A Spark of Calcium in Nerve Terminal

In rapid synapses, the rate of Ca^{2+} entry is highest at the end of depolarization. Therefore, a substantial part of the synaptic delay corresponds to the duration of the presynaptic action potential (Llinas et al., 1981). The spark of free Ca^{2+} is extremely brief and restricted to nanodomains situated at the inner mouth of Ca^{2+} channels (Llinas et al., 1992). At these spots, Ca^{2+} concentration reaches high levels explosively and then decays with a time constant as fast as 300–400 µs (Yazejian et al., 2000).

The Ca^{2+} spark is terminated first by a rapid process and then by slower mechanisms, probably Ca^{2+}-ATPase, which further reduces Ca^{2+} to submicromolar range (Castonguay and Robitaille, 2001). Although diffusion and binding to cytosolic proteins should contribute to the initial phase of Ca^{2+} buffering, a Ca^{2+}/H^{+} antiport ensures rapid Ca^{2+} sequestration into vesicles (Gonçalves et al., 1998). The Ca^{2+}/H^{+} antiport has a low affinity for Ca^{2+} (K_m = 217 µ*M*) and is energized by a pre-established proton gradient. Inhibition of the vesicular Ca^{2+}/H^{+} antiport increases the duration of phasic transmitter release (*see* Cordeiro et al., this volume).

Calcium enters terminals during a brief stimulation, transiently accumulates in synaptic vesicles, and returns to resting level during the minutes following the burst (Fig. 1A). Although the total number of vesicles does not change during and after the stimulation, the proportion of vesicles containing a calcium spot significantly increases after the tetanus and subsequently returns to control value, in parallel to the decline of the extra calcium accumulated in the terminals (Fig. 1A,C) (Babel-Guérin, 1974; Parducz and Dunant, 1993; Parducz et al., 1994). Transient Ca accumulation in synaptic vesicles after activity was also demonstrated in cholinergic and other synapses (Parducz et al., 1987; Buchs et al., 1994).

Calcium is subsequently cleared from vesicles, probably by exocytosis. The density of vesicle openings does not increase at the very moment of synaptic transmission (Muller et al., 1987) but, as seen in Fig. 1C,D, clearly rises during the minutes following tetanus (Parducz et al., 1994). Such a delayed exocytosis was frequently reported after stimulation of various synapses (*see* refs. in Dunant, 2000). In brief, opening of the presynaptic voltage-gated channel switches on a small Ca^{2+} spark that is extinguished mainly under the action of a vesicular Ca^{2+}/H^{+} antiport. A variety of other processes regulates presynaptic calcium homeostasis on a slower time scale.

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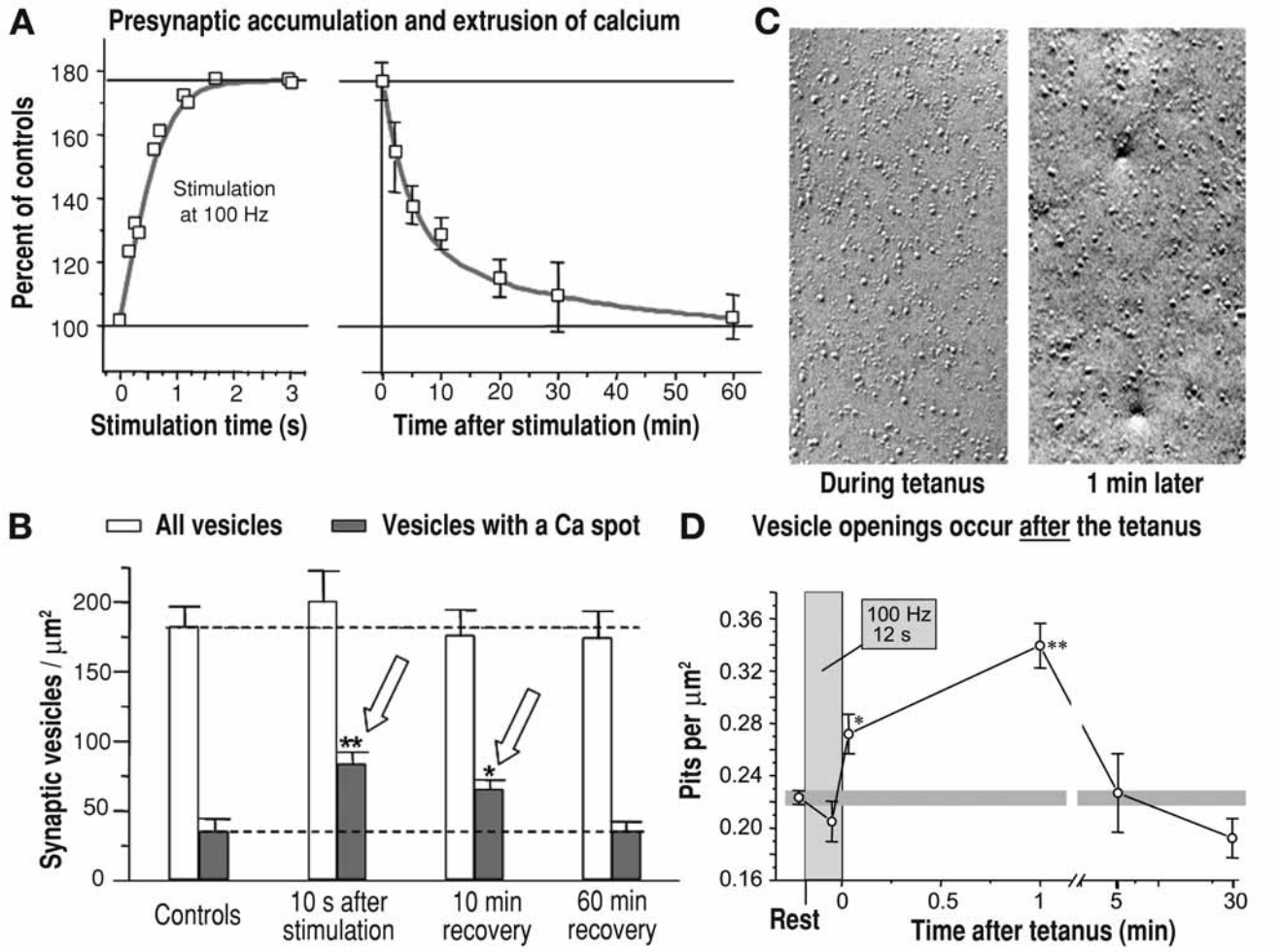

Fig. 1. Transient Ca accumulation in synaptic vesicles. **(A)** Extra-accumulation of presynaptic Ca at the *Torpedo* nerve-electrocyte junction during stimulation at 100 Hz for 12 s and the subsequent decay after activity. **(B)** Transient increase in the number of synaptic vesicles exhibiting a Ca deposit after a similar stimulation protocol. **(C)** Freeze-fracture replicas of the presynaptic membrane cryofixed during, and at 1 min after, stimulation. Note the late occurrence of large pits (vesicle openings). **(D)** Quantification of data from a series of similar experiments: The occurrence of vesicle openings culminates 1 min after stimulation.

A Spark of Acetylcholine in the Synaptic Cleft

The time lag between the presynaptic Ca^{2+} spark and the onset of the postsynaptic current is incredibly short—approx 250 µs (Llinas et al., 1981; Yazejian et al., 2000). One candidate molecule fulfills requirements for such an abrupt release, mediatophore, a homo-oligomer of approx 220 kDa, made up of identical 15- to 16-kDa proteolipid subunits, isolated by Israël et al. (1986) using release from acetylcholine (ACh)-loaded proteoliposomes as a functional assay. Cloned and sequenced in *Torpedo* (Birman et al., 1990), mediatophore turned out to be a member of a proteolipid family that includes the c-subunit of the membrane sector of vesicular (V)-ATPase (Nelson and Harvey, 1999).

Mediatophore, reconstituted into liposomes, oocytes, or cell lines, enables the preparations to release ACh in response to a Ca^{2+} challenge (Israël et al., 1986; Cavalli et al., 1993; Falk-Vairant et al., 1996). Release in these reconstituted systems mimics natural nerve terminals in all aspects, including the production of multimolecular quanta (Fig. 2A) (Katz, 1969; Falk-Vairant et al., 1996; Bloc et al., 1999).

Mediatophore activation is thus the key process in triggering the synaptic ACh spark. Termination of that ACh spark is well-known: At nerve-muscle and nerve-electroplaque junctions, cholinesterase

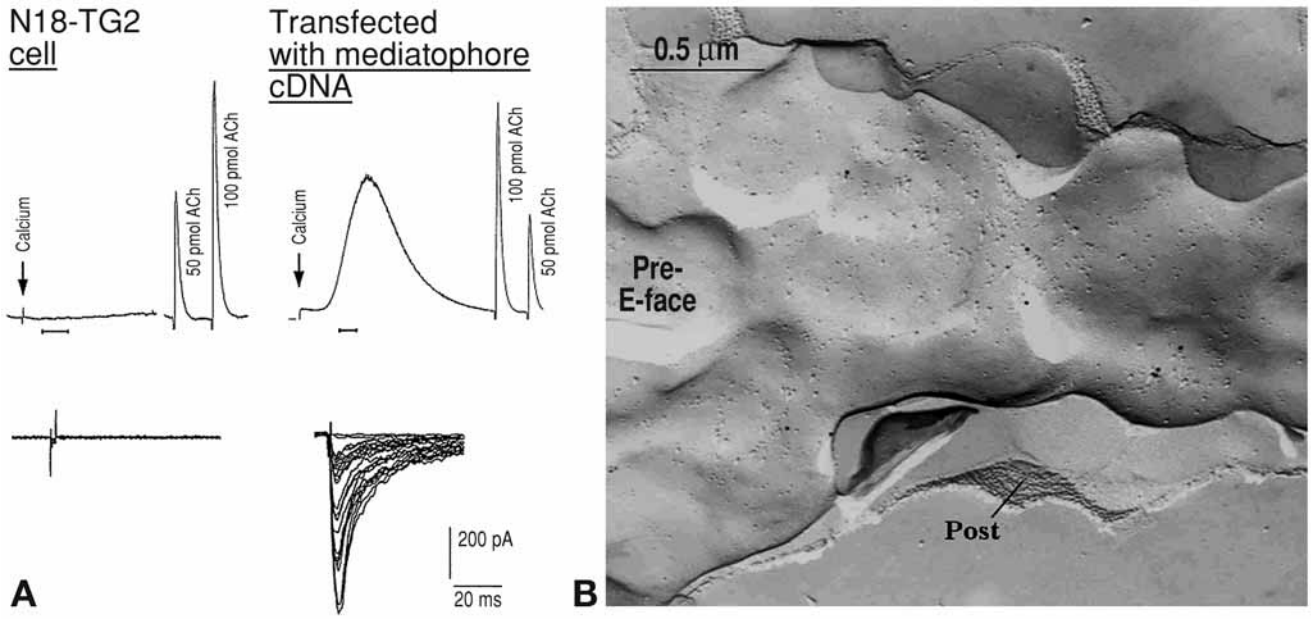

Fig. 2. **(A)** Induction of quantal ACh release by transfection of mediatophore cDNA. ACh release was measured biochemically (upper traces) or electrophysiologically in real time by using a *Xenopus* myoball as a sniffer cell (lower traces) from N18-TG2 cells prefilled with ACh. Transfection of *Torpedo* mediatophore enables release of ACh in a quantal and Ca^{2+}-dependent manner. **(B)** Nerve terminal (External leaflet [E-face] of presynaptic membrane) cryofixed during transmitter release, and showing immunogold decoration of mediatophore molecules. Post: fragments of the receptor-rich postsynaptic membrane.

concentrated in the cleft leaves little chance of ACh molecules hitting postsynaptic receptors twice. In other cholinergic synapses, diffusion out of the cleft seems to be the main process governing the decay of local ACh concentration.

Generalization and Conclusions

The mediatophore story is apparently straightforward: no mediatophore, no Ca^{2+}-dependent ACh release, no quanta. It is also in line with previous findings. First, the cytosolic pool of ACh is preferentially used and renewed on stimulation, whereas vesicular ACh remains firmly bound and is mobilized only after prolonged activity (Israël et al., 1979; Dunant, 1986). This remarkable stability of vesicular ACh is explained by strong binding to the intravesicular matrix (Reigada et al., 2003). Also, transmitter release is accompanied, within milliseconds, by the fleeting occurrence of a population of large intramembrane particles in the presynaptic membrane (Garcia-Segura et al., 1986; Muller et al., 1987). Figure 2B shows a presynaptic membrane that was cryofixed during the passage of a single nerve impulse and gold-labeled by anti-mediatophore antibody. The proteolipid is always found in close proximity to a large particle.

Unexpectedly, the 16-kDa proteolipid recently was shown to be involved in multiple functions (Fig. 3), in addition to its traditional role in vesicle acidification as part of the Vo sector of V-ATPase (Nelson and Harvey, 1999). In yeast, it is needed for the final, Ca^{2+}-dependent step of vesicle fusion, which forms a fusion pore before complete coalescence of membranes (Peters et al., 2001). Another component of Vo that is also present at the presynaptic plasma membrane (Morel et al., 2003), the a-subunit, seems required for transmission (Hiesinger et al., 2005).

In brief, rapid cholinergic transmission utilizes a chain of fast processes where the vesicular Ca^{2+}/H^{+} antiport and mediatophore play a central role, besides voltage- and ligand-activated ion channels. Modulation of transmission and maintenance of ACh and Ca^{2+} homeostasis are regulated by the more complex and slower mechanisms of the presynaptic apparatus.

"Science is really about the discovery of things that are not obvious. They are only obvious afterwards!" (A. S. V. Burgen).

Acknowledgments

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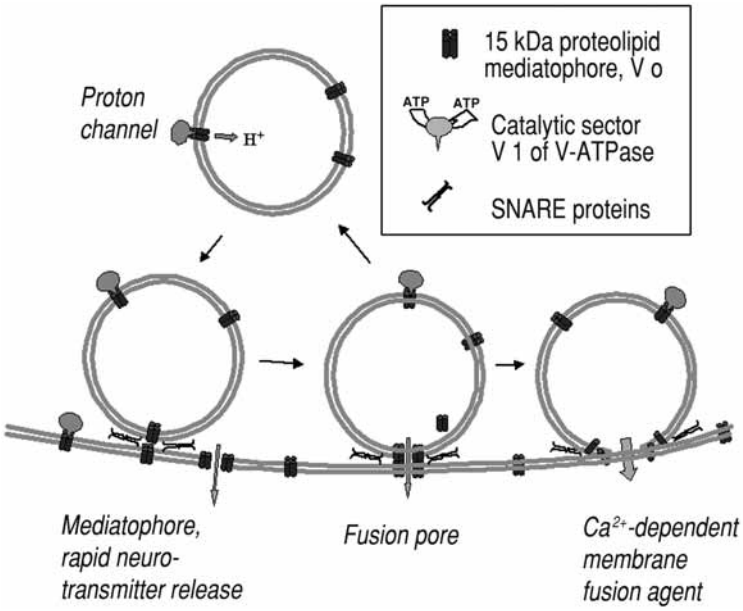

Fig. 3. Multiple functions of the 15- to 16-kDa proteolipid: (1) Proton channel, as the membrane sector (Vo) of V-ATPase; (2) mediatophore in the presynaptic plasma membrane involved in rapid transmitter release; (3) fusion pore for "kiss-and-run" and other secretions; (4) general Ca^{2+}-dependent agent in various types of membrane fusion; and (5) component of certain gap junctions (not shown).

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